Advanced features in Boolean models

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Motivation

We've seen how ODE models can incorporate the following features:

- bistability,
- dilution of protein concentation due to cellular growth,
- degradation (or decay) of protein concentration,
- time-delays due to cellular processes.

In this section, we'll see how Boolean models can incorporate these as well.

Bistability in Boolean networks

For bistability to exist, we need to be able to describe three levels of lactose: high, medium, and low.

In a Boolean network framework, one way to do this is to add variable(s).

Medium levels of lactose

Introduce a new variable L_m meaning "at least medium levels" of lactose. Clearly, $L = 1$ implies $L_m = 1$.

- High lactose: $L = 1$, $L_m = 1$.
- Medium lactose: $L = 0$, $L_m = 1$.
- Low lactose levels: $L = 0$, $L_m = 0$.

We can ignore any state for which $L = 1$, $L_m = 0$.

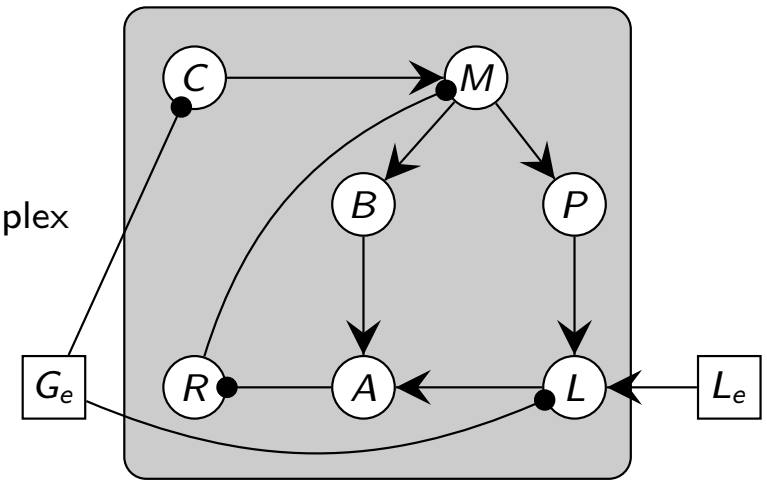
Since β-galactosidase converts lactose into allolactose, it makes sense to add a variable A_m to differentiate between high, medium, and low levels of allolactose.

It's not necessary, but we will also introduce R_m so we can speak of medium levels of the repressor protein.

A Boolean network model of the *lac* operon

Consider the following Boolean network model, which was published in Veliz-Cuba / Stigler (2011).

$M =$ mRNA
$P =$ *lac* permease
$B =$ β-galactosidase
$C =$ cAMP-CAP complex
$R =$ repressor protein
$L =$ lactose
$A =$ allolactose
$G =$ glucose



$$\begin{cases} f_M = \overline{R} \wedge \overline{R_m} \wedge C \\ f_P = M \\ f_B = M \\ f_C = \overline{G_e} \\ f_R = \overline{A} \wedge \overline{A_m} \\ f_{R_m} = (\overline{A} \wedge \overline{A_m}) \vee R \\ f_A = L \wedge B \\ f_{A_m} = L \vee L_m \\ f_L = \overline{G_e} \wedge P \wedge L_e \\ f_{L_m} = \overline{G_e} \wedge ((L_{em} \wedge P) \vee L_e) \end{cases}$$

Comments

- The shaded region represents the cell.
- Circles denote variables, and squares denote parameters.
- The subscript e denotes extracellular concentrations.
- The subscript m denotes medium concentration.

Analyzing our Boolean network

Now, we need to find the fixed point(s) for all six possible parameter vectors, (G_e, L_e, L_{em}).

We can disregard the cases where $L_e = 1$ and $L_{em} = 0$.

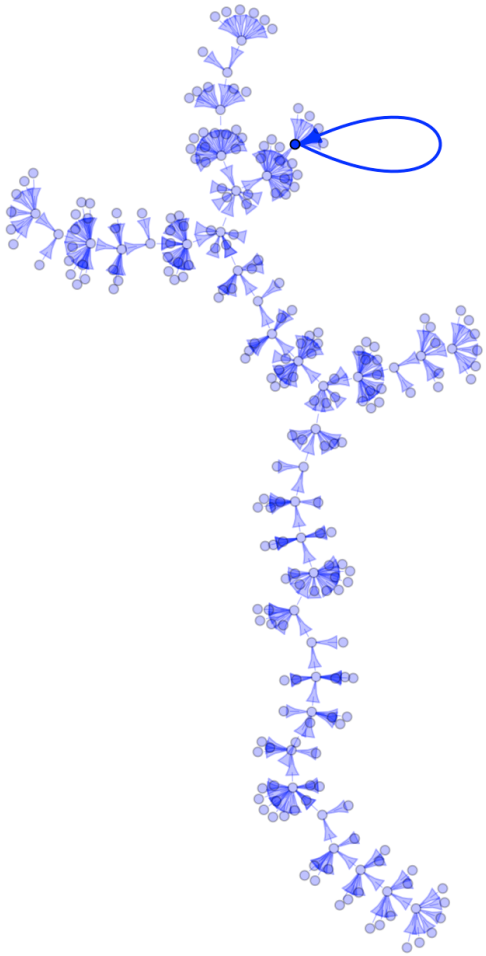
Here are several (freely available) ways we can analyze systems like this:

- Use the BoolNet package in R to compute the fixed points, limit cycles, or plot the phase space. (Lots of capabilities.)
- Use a computer algebra package (Singular, Macaulay2, Sage) to convert the functions into polynomials, and compute the fixed points using Gröbner bases.
- Use Cyclone in AlgoRun to nicely visualize the phase space with the nodes labeled as Boolean strings. (Need to convert to polynomials first.)
- Use the GINsim (Gene Interaction Network simulation) software to compute the fixed points and visualize the phase space.

All of these have their advantages and disadvantages.

Fixed point analysis and bistability

Here is the phase space with $(G_e, L_e, L_{em}) = (0, 0, 1)$, generated with BoolNet.



```
> print(getBasinOfAttraction(lacAttractorsBistable,2))
     State      Next state  Attr. basin  # trans. to attr.
1101001001 =>   1111000101            2                  1
1111001001 =>   1111000101            2                  1
1101000101 =>   1111000101            2                  1
1111000101 =>   1111000101            2                  0
1101001101 =>   1111000101            2                  1
1111001101 =>   1111000101            2                  1

Genes are encoded in the following order: M P B C R Rm A Am L Lm
```

Fixed point analysis and bistability

Computing the fixed point(s) for the other 5 initial conditions is an easy task for a computer.

(G_e, L_e, L_{em})	M	P	B	C	R	R_m	A	A_m	L	L_m	operon
$(1,0,0)$ $(1,0,1)$ $(1,1,1)$	0	0	0	0	1	1	0	0	0	0	OFF
$(0,0,0)$	0	0	0	1	1	1	0	0	0	0	OFF
$(0,1,1)$	1	1	1	1	0	0	1	1	1	1	ON
$(0,0,1)$	0	0	0	1	1	1	0	0	0	0	OFF
	1	1	1	1	0	0	0	1	0	1	ON

Suppose glucose or lactose are both absent ($L_e = L_{em} = G_e = 0$), so the operon is OFF:

$$(M, P, B, C, R, R_m, A, A_m, L, L_m) = (0,0,0,1,1,1,0,0,0,0).$$

Now, let's change L_{em} from 0 to 1, increasing lactose to medium. This is in the basin of the "low" bistable fixed point, so the operon remains OFF.

Conversely, suppose lactose concentration is high ($L_e = L_{em} = 1$), and so the operon is ON:

$$(M, P, B, C, R, R_m, A, A_m, L, L_m) = (1,1,1,1,0,0,0,1,0,1).$$

Now, let's change L_e from 1 to 0, reducing lactose levels to medium. This is in the basin of the "high" bistable fixed point, so the operon remains ON.

Dilution and degradation

Suppose Y regulates the production of X.

Assume $Y(t) = 1$ implies $X(t+1) = 1$. (activation takes 1 step).

Generally, the loss of X due to dilution and degradation takes several steps.

Introduce new variables $X_{\text{old}(1)}, X_{\text{old}(2)}, \ldots, X_{\text{old}(n)}$.

Properties

(i) If $Y(t) = 0$ and $X(t) = 1$, then $X_{\text{old}(1)}(t+1) = 1$. (*"$X$ has been reduced once by dilution & degradation."*)

(ii) If $Y(t) = 0$ and $X_{\text{old}(i-1)}(t) = 1$, then $X_{\text{old}(i)}(t+1) = 1$. (*"$X$ has been reduced i times by dilution & degradation."*)

(iii) The number of "old" variables is determined by the number of timesteps required to reduce $[X]$ below the discretation threshold.

Thus, $X(t+1) = 1$ when either of the following holds:

- $Y(t) = 1$ (new amount will be produced by $t+1$),
- $X(t) \wedge \overline{X_{\text{old}(n)}(t)} = 1$ (previous amounts of X still available).

$$X(t+1) = Y(t) \vee \left(X(t) \wedge \overline{X_{\text{old}(n)}(t)} \right)$$

Time delays

Cellular processes such as transcription and translation are not instantaneous.

Time delays

Suppose R regulates production of X, delayed by time τ (n steps).

Introduce new variables $R_1, R_2, \ldots, R_n$, with transition functions:

$$
\begin{aligned}
R_1(t+1) &= R(t) \\
R_2(t+1) &= R_1(t) \\
R_3(t+1) &= R_2(t) \\
&\vdots \\
R_n(t+1) &= R_{n-1}(t) \\
X(t+1) &= R_n(t)
\end{aligned}
$$

Estimating constants for our Boolean model

3-variable ODE model of the *lac* operon (Yildirim and Mackey, 2004)

Let $M(t) =$ mRNA, $B(t) = \beta$-galactosidase, and $A(t) =$ allolactose (concentrations), respectively.

$$\begin{aligned}
\frac{dM}{dt} &= \alpha_M \frac{1 + K_1(e^{-\mu\tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu\tau_M} A_{\tau_M})^n} - \widetilde{\gamma_M} M \\
\frac{dB}{dt} &= \alpha_B e^{-\mu\tau_B} M_{\tau_B} - \widetilde{\gamma_B} B \\
\frac{dA}{dt} &= \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \widetilde{\gamma_A} A
\end{aligned}$$

We need to estimate these rate constants and time delays from the literature.

- Time delays: $\tau_M = .10$ min, $\tau_B = 2.00$ min.
- Degradtion rates are harder to determine experimentally, and they vary widely in the literaure. Sample values:

$$\begin{cases}
\gamma_A = .52 \text{ min}^{-1}, \quad .0135 \text{ min}^{-1}, \quad .00018 \text{ min}^{-1} \\
\gamma_B = .00083 \text{ min}^{-1}, \\
\gamma_M = .411 \text{ min}^{-1}, \\
\mu \in (.0045,\ .0347)
\end{cases}$$

Estimating constants for our Boolean model

Approach

We'll select "middle of range" estimates for the rate constants:

- $\mu = .03\ \text{min}^{-1}$,
- $\gamma_A = .014\ \text{min}^{-1} \implies \widetilde{\gamma_A} = \gamma_A + \mu = .044$,
- $\gamma_B = .001\ \text{min}^{-1} \implies \widetilde{\gamma_B} = \gamma_B + \mu = .031$,
- $\gamma_M = .411\ \text{min}^{-1} \implies \widetilde{\gamma_M} = \gamma_M + \mu = .441$.

Degradation is assumed to be exponential decay: $x' = -kx$ implies $x(t) = Ce^{-kt}$.

The half-life is the time t such that:

$$x(t) = Ce^{-kt} = .5C \implies e^{-kt} = .5 \implies -kt = \ln \tfrac{1}{2} \implies \boxed{t = \frac{\ln 2}{k}}$$

Half-lives

- $\widetilde{h_A} = \frac{\ln 2}{\widetilde{\gamma_A}} = 15.753$ (*approx. 1 time-step to decay*)
- $\widetilde{h_B} = \frac{\ln 2}{\widetilde{\gamma_B}} = 22.360$ (*approx. 2 time-steps to decay*)
- $\widetilde{h_M} = \frac{\ln 2}{\widetilde{\gamma_M}} = 1.5$ (*approx. 0 time-steps to decay*)

A Boolean model incorporating dilution and degradation

Model assumptions

- Variables are M, B, A.
- Glucose absent. Intracellular lactose present, two parameters: L and L_m.
- Time-step $\approx$ 12 min.
- *Ignore* (all $\ll 12$): $\tau_M = .10$ min, $\tau_B = 2$ min, $\widetilde{h_M} = 1.572$ min.
- Introduce variables for dilution and degradation:
  - A_{old} (since $\widetilde{h_A} \approx 15.8 \approx 1$ timestep)
  - B_{old}, $B_{\text{old}(2)}$ (since $\widetilde{h_B} \approx 22.4 \approx 2$ timesteps)

Proposed model

$$f_M = A$$
$$f_A = (B \wedge L_m) \vee L \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right)$$
$$f_{A_{\text{old}}} = \left((\overline{B} \vee \overline{L_m}) \wedge \overline{L}\right) \wedge A$$
$$f_B = M \vee \left(B \wedge \overline{B_{\text{old}(2)}}\right)$$
$$f_{B_{\text{old}(1)}} = \overline{M} \wedge B$$
$$f_{B_{\text{old}(2)}} = \overline{M} \wedge B_{\text{old}(1)}$$

Most of the functions should be self-explanatory.

A Boolean model incorporating dilution and degradation

Justification for f_A

$$f_A = (B \wedge L_m) \vee L \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right)$$

There are 3 ways for allolactose to be available at $t+1$:

(i) β-galactosidase and at least medium levels of lactose are present;

(ii) high levels of lactose (assume basal concentrations of β-galactosidase);

(iii) Enough allolactose is present so that it's not degraded below the threshold, *and* no β-galactosidase is present.

Let's write our model into polynomials form, with parameters (L, L_m) and variables $(x_1, x_2, x_3, x_4, x_5, x_6) = (M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)})$:

$$f_M = A$$
$$f_A = (B \wedge L_m) \vee L \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right)$$
$$f_{A_{\text{old}}} = \left((\overline{B} \vee \overline{L_m}) \wedge \overline{L}\right) \wedge A$$
$$f_B = M \vee \left(B \wedge \overline{B_{\text{old}(2)}}\right)$$
$$f_{B_{\text{old}(1)}} = \overline{M} \wedge B$$
$$f_{B_{\text{old}(2)}} = \overline{M} \wedge B_{\text{old}(1)}$$

$$f_1 = x_2$$
$$f_2 = x_2(1+x_3)(1+x_4) + (L_m x_4 + L + x_4 L L_m) + x_2(1+x_3)(1+x_4)(L_m x_4 + L + x_4 L L_m)$$
$$f_3 = (1 + x_4 L_m)(1 + L)x_2$$
$$f_4 = x_1 + x_4(1 + x_6) + x_1 x_4(1 + x_6)$$
$$f_5 = (1 + x_1)x_4$$
$$f_6 = (1 + x_1)x_5$$

Using M2 and Cyclone to compute the fixed points (low lactose)

Let's use:

- M2 to convert our Boolean functions into polynomials, with parameters $(L, L_m) = (0, 0)$
- Cyclone to compute the phase space.

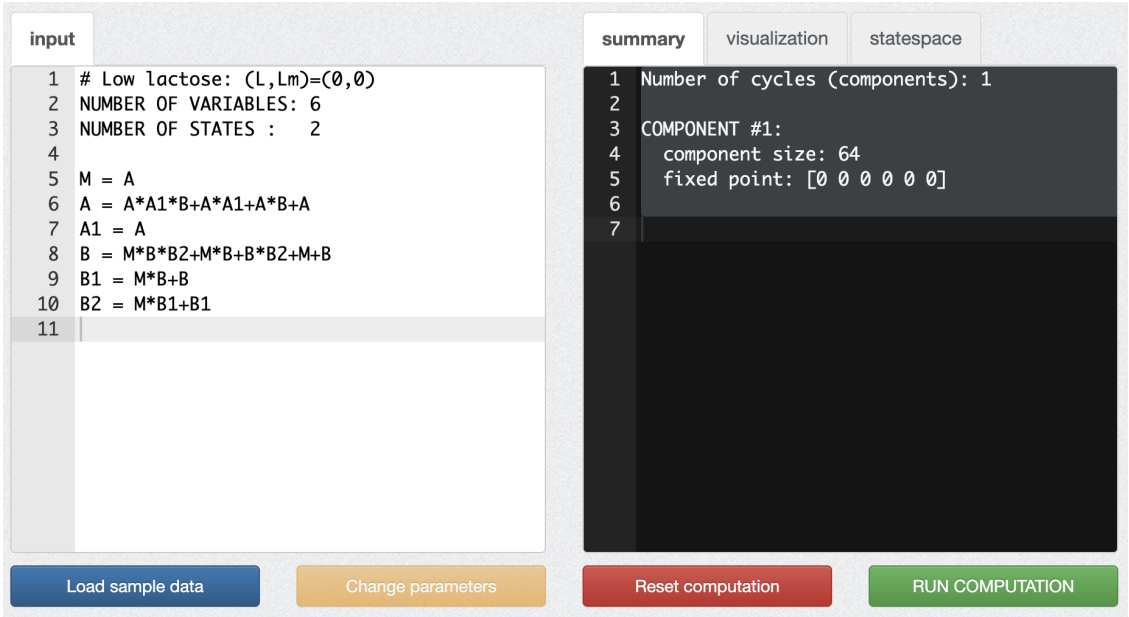
```
R = ZZ/2[M,A,A1,B,B1,B2];
I = ideal(M^2-M,A^2-A,A1^2-A1,B^2-B,B1^2-B1,B2^2-B2);
Q = R/I;

L = 0_Q;
Lm = 0_Q;

RingElement | RingElement :=(x,y)->x+y+x*y;
RingElement & RingElement :=(x,y)->x*y;

fM = A;
fA = (B & Lm) | L | (A & (1+A1) & (1+B));
fA1 = (((1+B) | (1+Lm)) & (1+L)) & A;
fB = M | (B & (1+B2));
fB1 = (1+M) & B;
fB2 = (1+M) & B1;

(fM, fA, fA1, fB, fB1, fB2)
```



The phase space has a unique basin of attraction with fixed point

$$(M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)}) = (x_1, x_2, x_3, x_4, x_5, x_6) = (0, 0, 0, 0, 0, 0).$$

This is exactly what we expected: the *lac* operon is OFF.

Using M2 and Cyclone to compute the fixed points (high lactose)

Let's use:

- M2 to convert our Boolean functions into polynomials, with parameters $(L, L_m) = (1, 1)$
- Cyclone to compute the phase space.

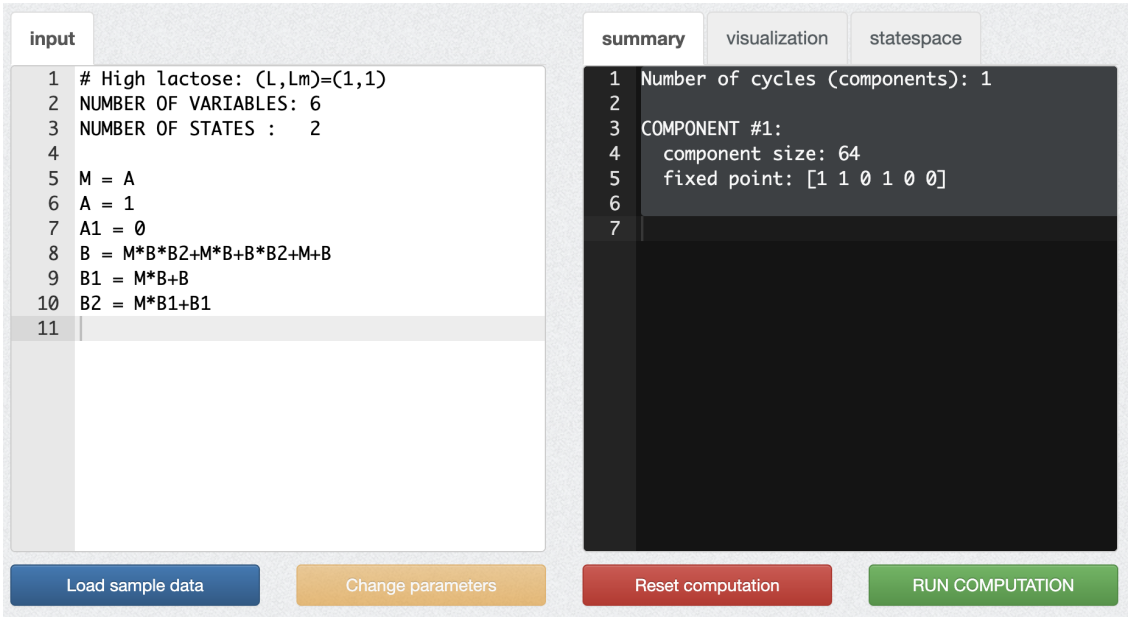
```
R = ZZ/2[M,A,A1,B,B1,B2];
I = ideal(M^2-M,A^2-A,A1^2-A1,B^2-B,B1^2-B1,B2^2-B2);
Q = R/I;

L = 1_Q;
Lm = 1_Q;

RingElement | RingElement :=(x,y)->x+y+x*y;
RingElement & RingElement :=(x,y)->x*y;

fM = A;
fA = (B & Lm) | L | (A & (1+A1) & (1+B));
fA1 = (((1+B) | (1+Lm)) & (1+L)) & A;
fB = M | (B & (1+B2));
fB1 = (1+M) & B;
fB2 = (1+M) & B1;

(fM, fA, fA1, fB, fB1, fB2)
```



The phase space has a unique basin of attraction with fixed point

$$(M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)}) = (x_1, x_2, x_3, x_4, x_5, x_6) = (1, 1, 0, 1, 0, 0)$$

This is exactly what we expected: the *lac* operon is ON.

Using M2 and Cyclone to compute the fixed points (medium lactose)

Let's do this one more time but with with parameters $(L, L_m) = (0, 1)$

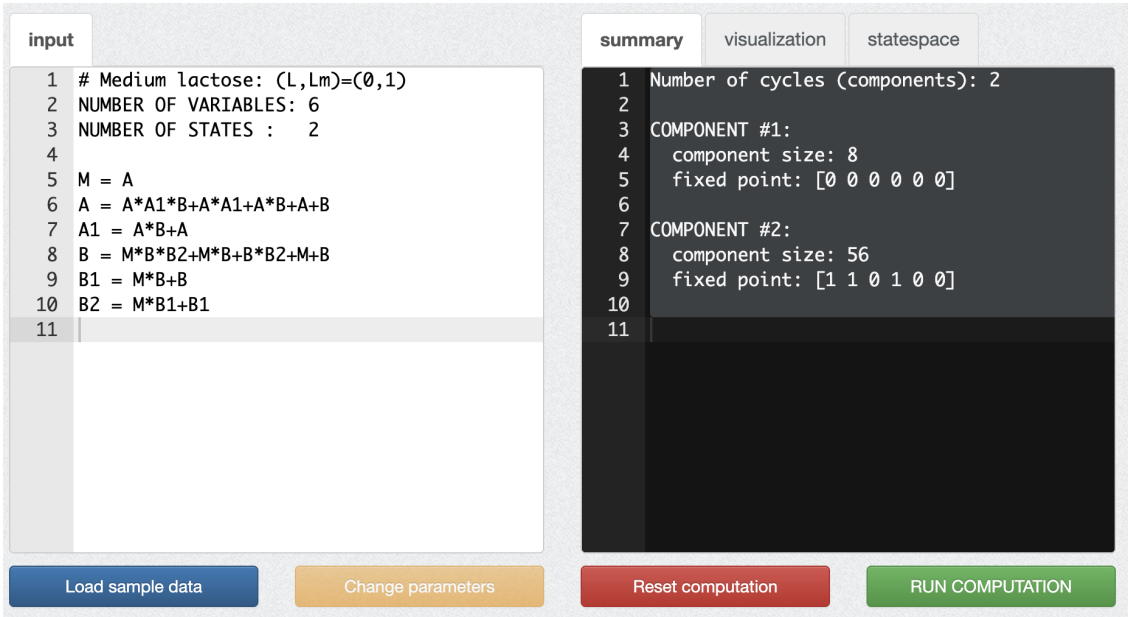
```
R = ZZ/2[M,A,A1,B,B1,B2];
I = ideal(M^2-M,A^2-A,A1^2-A1,B^2-B,B1^2-B1,B2^2-B2);
Q = R/I;

L = 0_Q;
Lm = 1_Q;

RingElement | RingElement :=(x,y)->x+y+x*y;
RingElement & RingElement :=(x,y)->x*y;

fM = A;
fA = (B & Lm) | L | (A & (1+A1) & (1+B));
fA1 = (((1+B) | (1+Lm)) & (1+L)) & A;
fB = M | (B & (1+B2));
fB1 = (1+M) & B;
fB2 = (1+M) & B1;

(fM, fA, fA1, fB, fB1, fB2)
```



The phase space has two basins of attraction, each with a fixed point:

$$(M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)}) = (x_1, x_2, x_3, x_4, x_5, x_6) = (0, 0, 0, 0, 0, 0), \text{ and } (1, 1, 0, 1, 0, 0).$$

Do you see how these are encoded in the following Gröber basis of $(f_i + x_i \mid i = 1, \ldots, 6)$?

```
( B2   B1   A1   A+B   M+B )
```

Fixed points of our model and bistability

Here is a table showing the fixed points of our model, depending on whether extracellular lactose levels are low, medium, or high.

Inducer level	L	L_m	M	B	$B_{\text{old}(1)}$	$B_{\text{old}(2)}$	A	A_{old}	operon
Low lactose	0	0	0	0	0	0	0	0	OFF
High lactose	1	1	1	1	0	0	1	0	ON
Medium lactose	0	1	0	0	0	0	0	0	OFF
Medium lactose	0	1	1	1	0	0	1	0	ON

Suppose lactose concentration is low ($L = L_m = 0$), and so the operon is OFF. The current state is

$$(M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)}) = (x_1, x_2, x_3, x_4, x_5, x_6) = (0, 0, 0, 0, 0, 0),$$

Now, let's change L_m from 0 to 1, increasing the lactose level to medium. We are now in the 3rd fixed point above, and so the operon is still OFF.

Conversely, suppose lactose concentration is high ($L = L_m = 1$), and so the operon is ON. The current state is

$$(M, A, A_{\text{old}}, B, B_{\text{old}(1)}, B_{\text{old}(2)}) = (x_1, x_2, x_3, x_4, x_5, x_6) = (1, 1, 0, 1, 0, 0),$$

Now, let's change L from 1 to 0, reducing the lactose level to medium. This takes us to the 4th fixed point above, and so the operon is still ON.

A Boolean model incorporating dilution & degradation, and time-delays

Instead of the a "middle value" ($.135\ \text{min}^{-1}$), let's choose the high estimate $\gamma_A = .52\ \text{min}^{-1}$.

This makes the half-life of A (which was $\widetilde{h_A} = 15.753$) much smaller:

$$\widetilde{h_A} = \frac{\ln 2}{\widetilde{\gamma_A}} = 1.260, \qquad \widetilde{h_B} = \frac{\ln 2}{\widetilde{\gamma_B}} = 22.360 \qquad \widetilde{h_M} = \frac{\ln 2}{\widetilde{\gamma_M}} = 1.5$$

In this case, let's choose a much smaller time-step (e.g., $t = 1$ min).

We can no longer ignore all of the time-delays, so we introduce the following new variables:

- M_1, M_2 to model the delayed effect (by $\tau_B = 2$ min) of mRNA on the production of β-galactosidase.
- A_1 to model the delayed action of A on the production of mRNA by $\tau_M = .1$ min.

We will use the following new variables to model dilution & degradation:

- M_{old} since $\widetilde{h_M} = 1.5$ is approximately 1 time-step.
- A_{old} since $\widetilde{h_A} = 1.26$ is approximately 1 time-step.
- $B_{\text{old}(1)}$, $B_{\text{old}(2)}$ since loss of β-galactosidase is slower.

Remark

We really should use more variables, e.g., $B_{\text{old}(1)}, B_{\text{old}(2)}, \ldots, B_{\text{old}(22)}$ to accurately track the loss of β-galactosidase. However, we will argue shortly why this won't matter.

A Boolean model incorporating dilution & degradation, and time-delays

Proposed model

$$f_M = A_1 \vee (M \wedge \overline{M_{\text{old}}}) \qquad f_{A_1} = A$$
$$f_{M_1} = M \qquad f_{A_{\text{old}}} = \left((\overline{B} \vee \overline{L_m}) \wedge \overline{L}\right) \wedge A$$
$$f_{M_2} = M_1 \qquad f_B = M_2 \vee \left(B \wedge \overline{B_{\text{old}(2)}}\right)$$
$$f_{M_{\text{old}}} = \overline{A_1} \wedge M \qquad f_{B_{\text{old}(1)}} = \overline{M_2} \wedge B$$
$$f_A = (B \wedge L_m) \vee L \vee (A \wedge \overline{A_{\text{old}}} \wedge \overline{B}) \qquad f_{B_{\text{old}(2)}} = \overline{M_2} \wedge B_{\text{old}\,(1)}$$

Analysis of the long-term behavior of this model leads to similar results as the previous one.

Lactose	L	L_m	M	M_1	M_2	M_{old}	B	$B_{\text{old}(1)}$	$B_{\text{old}(2)}$	A	A_1	A_{old}
Low	0	0	0	0	0	0	0	0	0	0	0	0
High	1	1	1	1	1	0	1	0	0	1	1	0
Medium	0	1	0	0	0	0	0	0	0	0	0	0
Medium	0	1	1	1	1	0	1	0	0	1	1	0

A Boolean version of the 5-variable ODE model

5-variable ODE model (Yildirim and Mackey, 2004)

Let $M(t)$ = mRNA, $B(t)$ = β-galactosidase, $A(t)$ = allolactose, $P(t)$ = *lac* permease, $L(t)$ = lactose (concentrations). Extracellular lactose (L_e) is a parameter.

$$\begin{aligned}
\frac{dM}{dt} &= \alpha_M \frac{1 + K_1(e^{-\mu\tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu\tau_M} A_{\tau_M})^n} + \Gamma_0 - \widetilde{\gamma_M} M \\
\frac{dB}{dt} &= \alpha_B e^{-\mu\tau_B} M_{\tau_B} - \widetilde{\gamma_B} B \\
\frac{dA}{dt} &= \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \widetilde{\gamma_A} A \\
\frac{dP}{dt} &= \alpha_P e^{-\mu(\tau_B + \tau_P)} M_{\tau_B + \tau_P} - \widetilde{\gamma_P} P \\
\frac{dL}{dt} &= \alpha_L P \frac{L_e}{K_{L_e} + L_e} - \beta_{L_e} P \frac{L}{K_{L_e} + L} - \alpha_A B \frac{L}{K_L + L} - \widetilde{\gamma_L} L
\end{aligned}$$

We'll use the same estimates for degradation and delay constants as in the 3-variable model:

$$\mu = .03 \text{ min}^{-1}, \qquad \widetilde{\gamma_A} = \gamma + \mu = .044, \qquad \widetilde{\gamma_B} = \gamma + \mu = .031, \qquad \widetilde{\gamma_M} = \gamma + \mu = .441.$$

New degradation constants estimated at $\gamma_L = 0.0 \text{ min}^{-1}$, and $\gamma_P = .65 \text{ min}^{-1}$. Delay constant estimate is $\tau_P = .83$ min.

We need a new parameter to help distinguish high vs. medium extracellular lactose: L_{em}.

A Boolean version of the 5-variable ODE model

Model assumptions

- Variables are M, B, A, P, L.
- Glucose absent. Extracellular lactose present, two parameters: L_e and L_{em}.
- Ignore time-delays (Yildirim and Mackey showed that they do not affect bistability).
- Time-step $\approx$ 12 min.
- *Ignore* (all $\ll 12$): $\tau_M = .10$ min, $\tau_B = 2$ min, $\widetilde{h_M} = 1.572$ min.
- Introduce dilution & degradation variables: A_{old}, B_{old}, L_{old}, P_{old}.

Proposed model

$$f_M = A \vee (M \wedge \overline{M_{\text{old}}}) \qquad f_B = M \vee \left(B \wedge \overline{B_{\text{old}}}\right)$$

$$f_{M_{\text{old}}} = \overline{A} \wedge M \qquad f_{B_{\text{old}}} = \overline{M} \wedge B$$

$$f_A = (B \wedge L) \vee (L \wedge L_e) \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right) \qquad f_P = M \vee \left(P \wedge \overline{P_{\text{old}}}\right)$$

$$f_{A_{\text{old}}} = \left(\overline{B} \vee \overline{L}\right) \wedge \left(\overline{L} \vee \overline{L_e}\right) \wedge A \qquad f_{P_{\text{old}}} = \overline{M} \wedge P$$

$$f_L = ((P \wedge L_{em}) \vee L_e) \vee \left((L \wedge \overline{L_{\text{old}}}) \wedge (\overline{B} \wedge \overline{P})\right) \qquad f_{L_{\text{old}}} = \left((\overline{P} \vee \overline{L_{em}}) \wedge \overline{L_e}\right) \wedge L$$

A Boolean model incorporating dilution and degradation

Justification for f_A

$$f_A = (B \wedge L) \vee (L \wedge L_e) \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right)$$

There are 3 ways for allolactose to be available at $t+1$:

(i) β-galactosidase and lactose are present.

(ii) Internal lactose is present and the concentration of extracellular lacatose is high. This ensures that by time $t+1$, intracellular lactose concentration is high enough to find available trace amounts of β-galactosidase.

(iii) The concentration of allolactose is high enough that it won't be reduced below the threshold due to dilution & degradation, or to conversion (by β-galactosidase) to glucose & galctose.

Justification for f_L

$$f_L = ((P \wedge L_{em}) \vee L_e) \vee \left((L \wedge \overline{L_{\text{old}}}) \wedge (\overline{B} \wedge \overline{P})\right)$$

There are 3 ways for intracellular lactose to be available at $t+1$:

(i) *Lac* permease and extracellular lactose are available.

(ii) There are high levels of extracellular lactose available (even if *lac* permease level is low).

(iii) There is enough lactose in the cell that it won't be lost to dilution & degradaton, transport out, or conversion into allolactose (by β-galactosidase).

A Boolean model incorporating dilution and degradation

Model:

$$f_M = A \vee (M \wedge \overline{M_{\text{old}}}) \qquad f_B = M \vee \left(B \wedge \overline{B_{\text{old}}}\right)$$
$$f_{M_{\text{old}}} = \overline{A} \wedge M \qquad f_{B_{\text{old}}} = \overline{M} \wedge B$$
$$f_A = (B \wedge L) \vee (L \wedge L_e) \vee \left(A \wedge \overline{A_{\text{old}}} \wedge \overline{B}\right) \qquad f_P = M \vee \left(P \wedge \overline{P_{\text{old}}}\right)$$
$$f_{A_{\text{old}}} = \left(\overline{B} \vee \overline{L}\right) \wedge \left(\overline{L} \vee \overline{L_e}\right) \wedge A \qquad f_{P_{\text{old}}} = \overline{M} \wedge P$$
$$f_L = ((P \wedge L_{em}) \vee L_e) \vee \left((L \wedge \overline{L_{\text{old}}}) \wedge (\overline{B} \wedge \overline{P})\right) \qquad f_{L_{\text{old}}} = \left((\overline{P} \vee \overline{L_{em}}) \wedge \overline{L_e}\right) \wedge L$$

Fixed points:

Ext. Lactose	L_e	L_{em}	M	M_{old}	B	B_{old}	A	A_{old}	L	L_{old}	P	P_{old}
Low	0	0	0	0	0	0	0	0	0	0	0	0
High	1	1	1	0	1	0	1	0	1	0	1	0
Medium	0	1	0	0	0	0	0	0	0	0	0	0
Medium	0	1	1	0	1	0	1	0	1	0	1	0